REMARKS

This document is filed in reply to the Non-Final Office Action dated August 4, 2011. Applicants have amended claims 1 and 4 to more particularly point out and distinctly claim their invention. Support for "*Vibrio fischeri* [LuxR]" can be found in the Specification at, e.g., pages 2-3, bridging paragraph. Applicants have amended two paragraphs at pages 20 and 26 of the Specification to correct an obvious error (i.e., replacing "cell cytoplasmic membrane" with "cell outer membrane") and to capitalize a trademark (i.e., LUMICOM). No new matter is added.

Regarding the error correction, "[a]n amendment to correct an obvious error does not constitute new matter where one skilled in the art would not only recognize the existence of the error in the Specification, but also recognize the appropriate correction. *In re Oda*, 443 F.2d 1200, 170 USPQ 268 (CCPA 1971)." MPEP 2163 IB. Here, the use of the phrase "cell cytoplasmic membrane" at page 20, lines 18-19 of the Specification is a clear error for the reason that, in order for antibodies to bind to LuxR and LasR, the proteins must be present on the outer surface of the cell and not the cytoplasmic membrane. Moreover, disclosures for "cell outer membrane" appear in the Specification at, e.g., the paragraph bridging pages 2 and 3 and final paragraph at page 18.[1] In view of disclosures from the Specification and the context of the phrase, "one skilled in the art would not only recognize" that the phrase "cell cytoplasmic membrane" is an error, "but also recognize the appropriate correction," i.e., "cell outer membrane." Thus, the correction "does not constitute new matter."

Claims 1, 3-5, and 8-41 are pending in the application. Among them, claims 1, 3-5, 8, 30, and 41 are under examination. Reconsideration of this application is respectfully requested in view of the foregoing claim amendments and the following remarks.

Objections to Specification

At page 7 of the Office Action, the Examiner objected to the Specification for informality. Applicants have corrected the informality and request that the objection be withdrawn.

[1] More specifically, the paragraph bridging pages 2 discloses that "[t]he present invention is based on the discovery that the protein complexes involved in quorum sensing are found on the outer surface of the bacterial membrane during certain stages of growth;" the final paragraph at page 18 discloses that "the present invention shows that LuxR it is found on the outer surface of the outer membrane in pre-quorate and quorate cells," emphasis added.

Rejections over Ulrich *et al.* in light of Kolibachuk *et al*

Claims 1, 3, 5, 8, 30, and 41 remain rejected under 35 USC § 102(e) as allegedly being anticipated by U.S. PG-Pub 2004/0171020 by Ulrich *et al.* ("Ulrich") in light of Kolibachuk *et al.* (Journal. of Bacteriology Nov. 1993, vol. 175(22), pages 7307-7312; "Kolibachuk"). See the Office Action, page 2, item 2.

Applicants respectfully traverse. The essence of the Examiner's rejection is that both the instant patent Specification and the Kolibachuk reference describe that LuxR is associated with the cytoplasmic membrane. However, this assertion fails to appreciate the key distinction between the teachings of Kolibachuk and the instant patent Specification. In particular, while Kolibachuk describes that LuxR is associated with the inner surface of the cytoplasmic membrane and is therefore an intracellular protein, the instant patent Specification teaches that LuxR is in fact associated with the outer surface of the bacterial membrane and is therefore an extracellular protein.

More specifically, the Examiner referred to the passage in Kolibachuk at page 7311, col. 1, lines 16-20, which states (in full):

> "This leads to the suggestion that the N-terminal regulatory domain of LuxR is associated with the cytoplasmic membrane and the C-terminal, DNA-binding or transcription activator domain, which by itself is a cytoplasmic protein in E. coli, **extends into the cytoplasm of the cell**" (emphasis added)

Thus, Kolibachuk teaches that LuxR is associated with the surface of cytoplasmic membrane and is therefore an intracellular protein.

In contrast, the instant patent Specification teaches that LuxR is in fact associated with the outer surface of the bacterial membrane and is therefore an extracellular protein. To this end, the Examiner is particularly referred to the instant Specification in the paragraph bridging pages 2 and 3 and the final paragraph on page 18, fully supported by Figures 2 and 3 which demonstrate that LuxR and LasR are found on the outer surface of the cell membrane,[2] as anti-LuxR and anti-LasR respectively are able to bind the bacteria.

Indeed, at the priority date of the present invention (as exemplified by Kolibachuk), LuxR was believed to be an intracellular protein. It is the novel and entirely unexpected finding of the present inventors that LuxR is extracellular that forms the basis of the present invention.

[2] As addressed above, the erroneous reference to cytoplasmic membrane at page 20, line 19 of the instant Specification has been corrected, consistent with the remainder of the Specification.

In addition, it is reiterated that Ulrich neither teaches nor suggests an extracellular method for regulating quorum sensing in bacteria expressing LuxR or a homologue thereof, wherein the method comprises modulating the activation by a signalling molecule of LuxR or a homologue thereof by administering to said bacteria an antibody which specifically binds to LuxR or the homologue thereof. There is no basis in Ulrich in light of Kolibachuk for suggesting that the method disclosed in the present application would have worked. The generic disclosure in Ulrich relating to antibody formulations and the delivery of a composition to an animal therefore cannot anticipate the instant invention.

Thus, Applicants submit that claims 1, 3, 5, 8, 30, and 41 are novel over the cited references.

Rejections over Taga *et al.* in light of Kolibachuk or Bassler *et al.* in light of Raffa *et al.*

The Examiner further rejected one or more of claims 1, 3, 4, 8, 30, and 41 under 35 USC § 102 (b) as allegedly being anticipated by (i) U.S. PG-Pub 2003/0165932 by Taga *et al.* ("Taga") in light of Kolibachuk or (ii) US PG-Pub 20030148414 by Bassler *et al.*(" Bassler") in light of Raffa *et al.* (Journal of Pharmacology and Experimental Therapeutics, vol. 312(2), pages 417-423; "Raffa"). See the Office Action, pages 5 and 8. Applicants respectfully request that the Examiner reconsider this application in view of the above amendments and the following remarks.

First, the Examiner maintained her rejection that the claimed subject matter is anticipated by Taga in light of Kolibachuk. It is respectfully submitted that the Examiner appeared to have failed to appreciate the difference between the AI-2 system of *Vibrio harveyi* (involving LuxP and homologues thereof such as LsrR), which is the subject of Taga and the LuxI/LuxR (AHL) quorum sensing circuit of bacteria such as *Vibrio fischeri* (involving LuxR and homologues of LuxR such as LasR). In particular, the Examiner appeared to confuse disclosures of Taga relating to LsrR, with disclosure in the instant Specification of LasR as a homologue of LuxR. LsrR and LasR are <u>not</u> the same protein. More specifically, LsrR is a homologue of LuxP, which is involved in the AI-2 system (see, for example, Taga, page 29, col.1, paragraph [0325], which states *"Identification of LsrR: a protein responsible for AI-2 regulation of transcription of the lsr operon."*). In contrast, LasR is a homologue of LuxR, subject of the instant Specification and

which is involved in the AHL quorum sensing circuit. In view of the above remarks, the Examiner's ground for rejection over Taga in light of Kolibachuk is untenable.

Second, based on a further confusion between the AI-2 system of *Vibrio harveyi* and the LuxI/LuxR quorum sensing circuit of bacteria such as *Vibrio fischeri*, the Examiner raised a further rejection alleging that the subject matter of pending claims 1, 3, 8, 30 and 41 is anticipated by Bassler in light of Raffa. More specifically, the Examiner referred to the statement in Raffa at page 419, left column, last paragraph that *"It [AI-2 encoded by the luxS gene] binds to a LuxP protein (a LuxR homologue)(Coulthurst et al. 2002)."* However, Raffa provides no independent support LuxP being a homologue of LuxR. In support of this assertion, Raffa simply refers to Coulthurst *et al*. Yet, Coulthurst *et al*. contains no such statement. Thus, the Examiner's ground for rejection over Bassler in light of Raffa is also untenable.

More importantly, however, the teachings of Taga and Bassler are both irrelevant to the disclosure of the present application. In particular, both references relate to the autoinducer-2 (AI-2) system of *Vibrio harveyi* and homologous systems in other species (see Taga, page 8, paragraph [0133]; Bassler page 1, paragraph [0008]). Neither document relates to the LuxI/LuxR quorum sensing circuit of *Vibrio fischeri* and its homologues in other species, which is the subject of the instant application.

To that end, the Examiner is referred to Raffa (see particularly page 419, col. 1, section entitled "Autoinducers and Their Receptors"), which clearly distinguishes between three types of autoinducer pathways, namely the AHL(LuxI/LuxR) system of *V. fischeri* (subject of the instant application), the AIP system, and the AI-2 (LuxP) system of *V. harveyi*. Raffa states that "At least 25 species of Gram–negative bacteria (excluding *Vibrio harveyi* and *Myxococcus xanthus*) use "LuxI/LuxR-type" quorum sensing similar to that used by *V. fischeri*." Accordingly, the disclosures of Taga and Bassler relating to the autoinducer-2 (AI-2) system of *Vibrio harveyi* are both irrelevant to the disclosure of the present application.

Reference is also made to a review article by Federle *et al.* (Contrib Microbiol. 2009; 16: 18-32; copy attached as "Exhibit A"). This article clearly delineates the different autoinducer systems at page 2, paragraphs 1-3. In particular, it is stated that "LuxR of *V. harveyi* is not a member of the canonical *V. fischeri* LuxI/LuxR family of proteins; it does not bind HSLs." (see page 2, last paragraph, line 12; emphasis added).

The Examiner is further referred to the enclosed sequence alignments between LuxP of *V. harveyi* and LuxR of *V. harveyi* and *V. fischeri*, respectively. These alignments ("Exhibit B") show that, while the amino acid sequences of LuxP and LuxR of *V. harveyi* share some sequence homology, no such homology exists between LuxP of *V. harveyi* and LuxR of *V. fischeri*.

It further should be noted that LasR as recited in pending claim 4 does not refer to the transcriptional repressor LsrR in the Lsr system. The Lsr system is an AI-2 quorum sensing system present in *Salmonella typhimurium* and *Eschericha coli* which share some homology with the AI-2 system in *V. harveyi* (see Federle *et al.*, page 3, paragraphs 3 and 4). As pointed out above, this system is not related to the LuxI/LuxR quorum sensing circuit referred to in the present application.

It is clear from the claims and the description that LasR must be a homologue of *V. fischeri* LuxR. The enclosed sequence alignments further evidence that the LasR sequence of Fig. 1 of the Specification is identical to the amino acid sequence of the *P. aeruginoas* LasR protein and does not share any sequence homology with the LsrR protein of *S. typhimurium* (SEQ ID NO. 36 in Taga).

For the above reasons, Applicants submit that claims 1, 3, 4, 8, 30, and 41 are novel over cited reference and respectfully request that the Examiner withdraw the grounds for rejection.

CONCLUSION

In view of the foregoing remarks, Applicants believe that this application is in a condition for allowance and an early notice to this effect is earnestly solicited. If the Examiner does not believe that such action can be taken at this time or if the Examiner feels that a telephone interview is necessary or desirable, Applicants welcome the Examiner to call the undersigned at 609-895-7065. The USPTO is authorized to charge Deposit Account No. 50-1943 for any charges in connection with this matter.

Respectfully submitted,

Date: February 3, 2012

By: /Jianming Jimmy Hao/

Jianming Jimmy Hao, Ph.D., J.D.
Reg. No. 54,694
Fox Rothschild LLP
997 Lenox Drive, Building 3
Lawrenceville, NJ 08648-2311
Telephone: (609) 844-3020
Facsimile: (609) 896-1469
Attorney for the Applicants

Exhibit A

Published in final edited form as:
Contrib Microbiol. 2009 ; 16: 18–32. doi:10.1159/000219371.

Autoinducer-2-Based Chemical Communication in Bacteria: Complexities of Interspecies Signaling

Michael J. Federle
Department of Microbiology and Immunology, College of Medicine, University of Illinois at Chicago, and College of Pharmacy, Center for Pharmaceutical Biotechnology, Chicago, Ill., USA

Abstract

Cell-cell communication in bacteria, called quorum sensing, relies on production, release, and detection of signaling molecules, termed autoinducers. Communication enables populations of cells to synchronize gene expression and therefore behave as a group in a manner akin to cells in multicellular organisms. Most quorum-sensing systems allow communication within an individual species of bacteria. However, one autoinducer, called AI-2, is produced and recognized by many different bacterial species, indicating that some bacteria communicate across species boundaries. Current studies are aimed at discovering the role that AI-2 plays in gene regulation. Differential gene expression in response to AI-2 may cause bacterial behavioral changes, such as biofilm formation or transition to a pathogenic state. Interestingly, multiple mechanisms to detect AI-2 exist. These differences likely reflect variations in the role that AI-2 plays for different bacteria. Additionally, structural analyses of the AI-2 receptor in *V. harveyi* have provided insight into bacterial trans-membrane signal transduction. A further understanding of bacterial quorum-sensing processes may facilitate development of new technologies aimed at interfering with bacterial communication and virulence.

Bacteria, long thought to exist as asocial entities, are increasingly acknowledged to use communication mechanisms to coordinate behaviors within a population. Cell-to-cell communication in bacteria, also known as quorum sensing, relies on the ability of bacteria to produce, secrete, and detect small molecules, termed autoinducers, in their surrounding environment. Scores of examples have been characterized in which a particular bacterial species produces a specific autoinducer molecule that is recognized only by that species. In addition, a newly discovered signaling molecule, called AI-2, is produced by many species of bacteria and is capable of eliciting responses in different species of bacteria.

Quorum sensing allows control of behaviors on a community-wide level. Bacterial cells detect the buildup of secreted autoinducers and respond with programmed changes in gene expression. Bacterial activities regulated by quorum sensing include biofilm development, virulence factor regulation, bioluminescence induction, antibiotic production, sporulation, and competence initiation. The understanding that bacteria produce and respond to extracellular signals makes it possible to develop technologies aimed at obstructing bacterial communication systems. By interfering with these systems, it may be possible to alter the course of a bacterium's ability to harm humans, animals, and industrial processes. The discovery that a single type of signaling molecule, AI-2, contributes to the control of behaviors in diverse types of bacteria raises the possibility that a single drug or class of inhibitors may be able to target communication in many bacterial species.

Intra- and Interspecies Quorum Sensing

The ability of bacteria to produce and release signals to coordinate gene expression in a population was discovered in experimentally tractable bacteria whose quorum-sensing-

dependent behaviors were easily quantifiable. The first bacterial cell-cell communication system studied at the molecular level was that of *Vibrio fischeri* that produces density-dependent bioluminescence [1]. The *V. fischeri* quorum-sensing system has become the paradigm for Gram-negative bacterial communication networks (fig. 1a). Typically, *V. fischeri* and other Gram-negative quorum-sensing bacteria have a LuxI enzyme that produces an acylated homoserine lactone (AHL) autoinducer by reacting *S*-adenosyl methionine (SAM) with an acylated acyl-carrier protein [2]. Each species uses a unique LuxI enzyme to generate an AHL autoinducer with a particular acyl side-chain making these signals species-specific. The AHL molecules diffuse across the membrane into the surrounding environment. Detection of AHL autoinducers is accomplished by LuxR-type proteins which have the dual ability of binding AHLs and DNA. At high cell densities, i.e., at high autoinducer concentrations, LuxR binds to a cognate AHL and undergoes a conformational change allowing the LuxR-AHL complex to bind DNA and control transcription of target genes. In *V. fischeri*, one of the targets for LuxR regulation is the set of genes encoding luciferase, and thus, at high autoinducer concentrations, LuxR promotes the expression of bioluminescence. Many other Gram-negative bacterial species use analogous LuxI/LuxR-type circuits, but their target genes are variable. For example, *Pseudomonas aeruginosa* controls biofilm development and secretion of enzymes contributing to its pathogenesis, *Agrobacterium tumefaciens* and *Rhizobium leguminosarum* regulate transfer of plasmid DNA between cells, and *Serratia marcescens* controls sliding motility. Each of these organisms uses a unique LuxI/R pathway or set of LuxI/R pathways, each utilizing different AHLs to regulate the above and other behaviors.

In *Vibrio harveyi*, a bioluminescent bacterial species related to *V. fischeri*, the quorum-sensing circuitry is more complex (fig. 1b). *V. harveyi* relies on multiple autoinducers to regulate light production. The predominant autoinducer contributing to luciferase production is an AHL-denoted HAI-1 (*V. harveyi* autoinducer-1). HAI-1 is *N*-(3-hydroxybutanoyl)homoserine lactone and it is synthesized by LuxM. The HAI-1 receptor, LuxN, is a membrane-spanning histidine sensor kinase protein belonging to the well-conserved family of bacterial two-component signal transduction proteins.

Two-component histidine sensor kinases autophosphorylate by transferring phosphate from ATP to a conserved histidine residue located on the kinase. A phosphorylation cascade ensues, where phosphate passes from histidine (H1) to aspartic acid (D1), and phosphate can continue to a second histidine (H2) and second aspartic acid (D2), located on proteins connected to, or separate from, the kinase. Phosphorylation of the terminal aspartic acid generally causes activation of a DNA-binding response regulator protein. In the absence of HAI-1, LuxN autophosphorylates on the conserved H1 residue, which is then passed on to D1 located within the C-terminal domain of LuxN [3]. Phosphate is sequentially passed to H2 on LuxU, a phosphotransfer protein, and then on to D2 located on LuxO, the DNA-binding response regulator [4]. Phosphorylation of LuxO promotes the expression of genes encoding five small regulatory RNAs (sRNAs) [5]. These five sRNAs, together with the sRNA chaperone Hfq, bind to and block translation of the *luxR* mRNA. (LuxR of *V. harveyi* is not a member of the canonical *V. fischeri* LuxI/LuxR family of proteins; it does not bind HSLs.) The *V. harveyi's* LuxR protein directly activates transcription of luciferase and regulates nearly 100 other genes [6]. When *V. harveyi* reaches high cell density, HAI-1 concentrations are also high. When bound to autoinducer, the enzymatic activity of LuxN switches from kinase to phosphatase, and LuxN drains phosphate from LuxU and LuxO. Dephosphorylated LuxO is unable to activate transcription of sRNA genes, and consequently, LuxR expression is derepressed, and luciferase is expressed, and light is produced.

Interestingly, in the absence of the HAI-1 system, *V. harveyi* continues to control luciferase production as a function of cell population density, albeit at 1,000 times lower light production per cell. *V. harveyi* mutants were screened for those unable to regulate light production in a background lacking the HAI-1 system, and a new autoinducer detection system was discovered [7]. Two genes identified in the screen encode components of the autoinducer receptor. The first gene, *luxP*, encodes a periplasmic binding protein LuxP, similar to the *Escherichia coli* ribose-binding protein. The second gene, named *luxQ*, encodes a two-component histidine kinase similar to LuxN. A third gene, *luxS*, encodes the autoinducer synthase, LuxS, which produces a signal which was named, for lack of a specific chemical nature, AI-2.

V. harveyi integrates AI-2 information directly into the quorum-sensing pathway described above. LuxP and LuxQ work together as the AI-2 receptor complex (see structural details below). At low cell densities, when AI-2 concentrations are low, LuxQ acts as a kinase in a manner analogous to LuxN. Phosphate follows the typical H1 to D1 transfer on LuxQ itself, and is then passed on to H2 of LuxU and D2 of LuxO. When both HAI-1 and AI-2 concentrations are low, the kinase activities of both LuxN and LuxQ maximize LuxO-phosphate levels in the cell, generating high levels of the sRNAs. Consequently, little LuxR protein is made, and the cells do not produce light. The phosphorylation pathway reverses under high autoinducer concentrations. AI-2 binds to LuxP, switching LuxQ from kinase to phosphatase, lowering levels of LuxU-P and LuxO-P. Ultimately, LuxR is derepressed and light is produced.

The *luxS* gene exists in over half of all sequenced bacterial genomes. Importantly, species containing *luxS* genes generate and secrete AI-2 activity into their surroundings. One question that has emerged is whether AI-2 is an important communication signal in other bacteria, and if so, what do these bacteria control with AI-2 information? For *E. coli* and *Salmonella typhimurium*, AI-2 plays a regulatory role. In these bacteria, a set of genes called *lsr* (*luxS* regulated) are induced that encode components of the machinery used for the import and processing of AI-2 [8,9]. This transporter is so effective that levels of AI-2 are diminished to near background levels outside the cells.

The AI-2 receptor in the Lsr system, LsrB, is a periplasmic binding protein similar to *V. harveyi's* AI-2 receptor LuxP. While the overall fold of this family of proteins is highly conserved (the prototype being the ribose binding protein, RbsB), primary sequence similarity between LsrB, LuxP and RbsB is low. RbsB's similarity to LuxP and LsrB is 47 and 41%, respectively. However, LuxP is only 11% similar to LsrB. Low sequence similarity reflects the fact that each protein interacts with different types of downstream components. Instead of interacting with a histidine sensor kinase protein, as is the case for LuxP, the LsrB protein delivers AI-2 to an ABC transporter that imports AI-2 into the cytoplasm (fig. 1c). Transport of AI-2 is coupled to its phosphorylation and sequestration, which is dependent on LsrK kinase activity [9]. Phosphorylated AI-2 subsequently binds and deactivates the transcriptional repressor LsrR. Transcription increases of the genes encoding the Lsr transporter and modification enzymes used to import, modify, and degrade AI-2. This positive feedback is required for rapid induction of the Lsr system and removal of AI-2 from the environment.

To date, several studies have identified additional genes differentially regulated in response to AI-2 or LsrR/LsrK in both *E. coli* and *S. typhimurium* strains, including pathogenic species. However, the ability of the Lsr transporter to rapidly reduce AI-2 levels in the surrounding environment has led to the hypothesis that *E. coli* and *S. typhimurium* not only rely on AI-2 to identify their 'quorum', but also to diminish AI-2 levels to 'confuse' other species occupying the same environment. As proof of this principle, *E. coli* and *V. harveyi*

were grown together and the effects of this co-culture on *V. harveyi* luciferase production was monitored. In the mixed species set up, as *V. harveyi* population numbers increased, luciferase production was induced. However, as AI-2 concentrations climbed, *E. coli*, in turn, induced its Lsr transporter, and AI-2 levels in the co-culture dropped dramatically. The rate of light production from *V. harveyi* declined, and the cells' gene expression pattern was maintained at the level appropriate for cell densities 10- to 100-fold below their actual population number [9]. Few bacterial species exist in pure culture in nature, and it is likely that communication networks are subject to quorum-sensing eavesdropping and interference, as the Lsr studies imply.

AI-2 Identification

The LuxS protein does not to share sequence homology to LuxI-type autoinducer syntheses, and common extraction techniques used to purify AHLs from bacterial cultures proved unsuccessful for isolating AI-2 activity, suggesting that it was a novel chemical. Clues to LuxS's function and to AI-2's chemical nature came from the notion that LuxS may be involved in a pathway with other genes lying within close proximity on the chromosome. *luxS* was often found near genes involved in methionine metabolism and the reactive methyl cycle. In *Borellia burgdorferi*, for example, *luxS* is preceded in an operon by the genes *metK* and *pfs*. MetK catalyzes the production of SAM by combining adenosine with methionine (fig. 2). SAM is an essential coenzyme whose function is to provide methyl groups to numerous substrates, for example, in the biosynthesis of DNA, RNA, fatty acids, and proteins. SAM-dependent transmethylation reactions result in the release of *S*-adenosyl homocysteine (SAH) as a byproduct. SAH inhibits transmethylation reactions and is therefore toxic. The second gene found near *luxS* in *B. burgdorferi* was *pfs*. The Pfs enzyme detoxifies SAH by removing adenine and generating *S*-ribosyl homocysteine (SRH). Cells break down SRH into homocysteine and 4,5-dihydroxy-2,3-pentanedione (DPD); however, no enzyme had ever been identified to catalyze this reaction. Indeed, as predicted, LuxS catalyzes this final reaction. Thus, bacteria that use Pfs and LuxS to recycle SAM pools generate DPD as an additional byproduct, and DPD is responsible for AI-2 activity [10].

DPD and AI-2 Activity

Use of DPD as an intercellular signaling molecule is interesting from the standpoint that this molecule is inherently unstable. DPD spontaneously cyclizes to form two enantiomers, 2*S*, 4*S*-DHMF and 2*R*,4*S*-DHMF (fig. 3) [11]. Hydration of the ketone in aqueous solutions leads to formation of *S*- and *R*-THMF. The multiple derivatives of DPD raised the question: which of these isomers is recognized as AI-2 by bacteria? Because conversion between these molecules is spontaneous and fast, isolation or synthesis of each compound for biological activity testing was impossible. Thus, determining which forms of DPD are active came from biochemical and structural studies of various DPD receptors. Crystals of *V. harveyi's* LuxP protein containing the AI-2 ligand within the binding pocket showed that a borated adduct of the *S*-THMF molecule is the *V. harveyi* signal [12]. Consistent with this, *Vibrio* species are found in marine environments where borate is plentiful. In contrast, levels of borate are low in terrestrial environments, yet bacteria such as *E. coli* and *S. typhimurium* also produce and respond to AI-2. These findings support the idea that borated DPD may not be the only active form of the molecule. Indeed, crystal structures of LsrB, the AI-2 binding protein from *S. typhimurium* contained the non-borated *R*-THMF ligand [11].

Knowing the two AI-2 structures provided a mechanism by which interspecies communication occurs. The LuxS enzyme, regardless of bacterial species origin, is responsible for the production of the linear DPD molecule, which through spontaneous rearrangements, produces a pool of interconverting compounds. A molecule recognized by

one species can rearrange to become the signal recognized by another species. It remains to be seen whether forms of DPD other than the two now known are recognized as AI-2 signals, or whether other types of adducts can be made from DPD to generate new, unanticipated AI-2 molecules.

Environmental conditions affect the equilibrium of AI-2 molecules, and may contribute to a bacterium's ability to monitor its surrounding environment. For instance, *V. cholerae*, a bacterium that contains *luxS* and responds to AI-2, has a life cycle that alternates between marine environments and the human intestine. As discussed above, the predominant form of AI-2 in the ocean is *S*-THMF-borate. However, in the human gastrointestinal tract, where only trace amounts of borate are available, AI-2 likely exists only in non-borated forms. It is possible that *V. cholerae* distinguishes these niches by measuring the availability of borated AI-2. Therefore, in addition to population density, information encoded within AI-2 can also include the status of the surrounding environment. It is also possible that bacteria detect multiple forms of AI-2 using different protein receptors. If so, this would allow direct monitoring of different AI-2 forms and the proportions of each.

Discriminating between AI-2 Signaling Effects and LuxS Metabolic Roles

A large effort has been underway to test the roles that LuxS and AI-2 play in controlling gene regulation. *luxS* null mutations have been generated in different bacterial species, and subsequent studies have ranged in scope from measuring changes in expression of a specific gene of interest in some species, to whole genome transcription profiles in others. For instance, specific virulence factors have been monitored in *luxS* mutants of *Clostridium perfringens*, *Shigella flexneri*, *Actinobacillus actinomycetemcomitans* and *Streptococcus pyogenes* [13–16]. Likewise, whole genome expression profiles have been compared between wild-type strains and *luxS* mutant for many species, including *E. coli* W113 and *E. coli* O157:H7, *Neisseria meningitidis*,*Streptococcus pneumoniae*, and *Streptococcus mutans* [17–21]. Typically, large numbers of genes are differentially regulated in *luxS* mutants (ranging from <1 to 10% of the genome) with many of these genes being unresponsive to exogenously added AI-2. The inability to complement *luxS* mutants with artificially added autoinducer raises the question of whether *luxS* mutations affect gene regulation in an AI-2-dependent manner, or whether the ramification of *luxS* mutations stem from alterations in SAM, SAH, or SRH levels. Several possibilities in addition to disruption of the reactive methyl cycle may also explain the lack of ability to complement *luxS* phenotypes, including the appropriate timing of signal presentation, the percent of the population responding to the signal, the concentration of the signal, if the signal requires chemical modification, or if feedback regulation affects the ability of cells to respond to AI-2.

In some quorum-sensing systems, a temporary window of time exists during which cells are able to respond to extracellular signals. Studies of competence development, a quorum-sensing-controlled process in *S. pneumoniae*, have shown that only 15–30 min exist when cells develop and maintain maximum competence [22]. After this interval, the response to the competence-stimulating autoinducer rapidly decreases. Shut-down of competence is now partially understood to be controlled by degradation of transcription factors responsible for induction of the competent state [23]. This stands as a clear example that programs triggered by autoinducers can be heavily regulated processes, and precise temporal conditions must be met to generate full responses. In the case of AI-2, cells experiencing only the correct concentrations of autoinducer may also be critical for proper responses. Recent studies of biofilm formation within mixed bacterial cultures of *Streptococcus oralis* and *Actinomyces naeslundii* found that optimal AI-2 concentrations exist for biofilm formation, and above and below this concentration, biofilm development is decreased [24].

Effects of added autoinducers on a bacterial culture also may be difficult to observe when only a subpopulation of cells is responsive to the extracellular signal. In *Bacillus subtilis*, competence develops in only 10% of the population in response to the competence-inducing autoinducer ComX [25]. Without an easily tractable phenotype, like uptake of DNA, effects on gene expression via microarray or proteomic analysis, or other methods that measure the mean change of expression of target genes in the population, may be nearly impossible to observe.

When using AI-2 to artificially stimulate cells, another difficulty to be considered is that some species of bacteria in which *luxS* has been deleted may be locked in a state that is unresponsive to exogenously added autoinducer. In *E. coli* and *S. typhimurium*, as mentioned, AI-2 is imported into the cell by the Lsr transporter. Transport occurs quickly due to upregulation of the Lsr apparatus, and AI-2 is completely removed from the surrounding environment in <2 h. In *luxS* mutants, exogenously supplied AI-2 requires approximately 60 min longer than in the wild type for proper induction of the Lsr system [8]. The response is possible only because a secondary, promiscuous transporter is capable of importing some AI-2, which can then kick-start the system. For species with other types of positive feedback regulation, it may be the case that deletion of *luxS* abolishes the basal level of AI-2 required to ensure that detection systems are primed for induction. Without this, cells are unable to respond to exogenously added AI-2.

Finally, phenotypes stemming from *luxS* mutations may be due to a combination of effects on the active methyl cycle as well as a loss of the signaling molecule AI-2. Genomic, proteomic, and metabalomic studies may provide initial clues for discriminating between, gene and protein level changes in response to AI-2 concentrations versus metabolic disturbances. A recent study in *S. mutans* that measured changes in gene expression using microarray analysis compared a wild-type strain to a *luxS* mutant and to a *luxS* mutant supplemented with chemically synthesized DPD. Over 500 genes (30% of genome) showed changes in gene expression in the *luxS* mutant, but only 59 of these genes were complemented by exogenously supplied DPD [21] .These results indicate that the large majority of genes whose expression changed as a result of the *luxS* mutation were not responsive to DPD under conditions tested. But the finding that a substantial number of genes are controlled by AI-2 provides footing for future studies to optimize conditions that may give insight to how *S. mutans* detects AI-2.

Further complications due to signal redundancy also complicate these analyses. In fact, the species in which AI-2 was discovered, *V. harveyi*, does not have an obvious *luxS* phenotype. A *V. harveyi luxS* mutant decreases light production about 100-fold when grown to high cell density as compared to wild type [26]. However, a 100-fold loss in light output accounts for less than 1% of the overall change in light production observed when AI-1 and AI-2 autoinducers are removed simultaneously.

A Structural Study of the AI-2 Receptor of *Vibrio harveyi*

One long-term goal of quorum-sensing studies is to identify compounds that can interfere with bacterial communication. To fully understand how small molecule antagonists interfere with cell-cell communication systems, the endogenous signaling molecules and the mechanisms by which information is relayed into the cell must be defined. The identification of the AI-2 signal, along with the identification of the proteins used to convert AI-2 information into target gene expression, makes such studies feasible. Of particular interest is to understand how AI-2 ligand binding in the periplasm leads to changes in enzymatic activity of the LuxQ sensor kinase in the cytoplasm.

Genetic studies had indicated that LuxP controls LuxQ's ability to switch from the kinase state (at low AI-2 concentrations) to the phosphatase state (at high AI-2 concentrations), because deletions of *luxP* cause LuxQ to remain locked in the kinase state [27]. To determine how conformational changes in LuxP control LuxQ enzymatic activity, combined structural and genetic studies were performed. *V. harveyi* LuxP and the periplasmic domain of LuxQ ($LuxQ_P$) were expressed in *E. coli* in the presence and absence of AI-2, and the protein complexes were purified and crystallized. LuxP and $LuxQ_P$ have two sites of interaction, and each is required for proper switching between kinase and phosphatase activities.

In protein complexes lacking AI-2, LuxP is in an open conformation and LuxP interacts with $LuxQ_P$ in a 1:1 stoichiometry forming a complex (the $LuxPQ_P$ monomeric subunit) [27]. Contacts between LuxP and $LuxQ_P$ occur at a location distal to the membrane, and this interaction is necessary for LuxQ kinase activity because deleting this region causes LuxQ to favor the phosphatase state in an AI-2-independent manner. The structure of the holo-LuxP-$LuxQ_P$ protein complex displayed two major differences from the apo-complex structure [28]. First, a second site of interaction between LuxP and LuxQ was evident. While the first site of interaction is identical to that observed in the apo-complex, the second site of interaction occurs because AI-2 binding induces a major conformational change in LuxP, and allows new contacts to form between LuxP and a second $LuxQ_P$subunit ($LuxQ_P'$). The second major structural difference in the holo-complex is the generation of an asymmetric dimer, consisting of two $LuxPQ_P$ monomers (designated $LuxPQ_P$-$LuxP'Q_P'$). When LuxP simultaneously contacts $LuxQ_P$ and $LuxQ_P'$, the complex is held together in an orientation that is asymmetric along the axis that is normal to the cytoplasmic membrane.

Interestingly, although a large conformational change occurs in each LuxP subunit upon binding AI-2, virtually no conformational change is observed in either $LuxQ_P$ subunit. It appears that signal propagation does not occur through intramolecular conformational changes that are passed across the membrane into the cytoplasmic domains of the protein. Rather, in the case of LuxQ, the relative orientation in which the periplasmic LuxQ subunits are arranged around the axis of symmetry determines the enzymatic state of the receptor.

The current model suggests that when AI-2 concentrations are low, LuxP is in an open conformation and only one contact is made with its paired LuxQ subunit. The orientation of LuxQ-LuxQ' dimers is determined by membrane-spanning and cytoplasmic domains of the protein and the dimer is situated in a symmetric orientation (fig. 4, left). Upon binding AI-2, LuxP undergoes large movements to close around the ligand. This movement reorients LuxP, making it possible to contact the $LuxQ_P'$ subunit. This contact drives rotation between the two LuxQ subunits into an asymmetric position (fig. 4, right). This asymmetric orientation must signal to the cytoplasmic domains to switch kinase activity off. The phosphatase activity of LuxQ is located in the C-terminal aspartic acid (D1)-containing domain, and is not dependent on autoinducer concentration. Therefore, the activity of LuxQ's kinase domain determines whether phosphate flows toward LuxU and LuxO or away from them. When the kinase is active, it overrides the phosphatase activity; when the kinase is inactivated, the phosphatase activity prevails, and phosphate is drained away from LuxU and LuxO.

The AI-2-LuxP-$LuxQ_P$ receptor complex is one of only a few bacterial signal transduction receptor complexes with structures that have been determined with and without bound ligand. Another such receptor system in which structural data are available is the chemotaxis receptor. Chemotaxis systems are used by bacteria to detect and subsequently move towards attractant and away from repellant molecules. Chemotaxis receptors measure changes in concentration of the attractants or repellants and relay this information to a signaling

network which controls the bacterium's swimming direction. These receptors cluster to form arrays of protein complexes in the membrane. Clustering of receptors is proposed to provide a mechanism to detect small fluctuations in attractant/repellant concentrations and then respond with large changes in swimming behavior. Unlike the chemotaxis receptors, the LuxP-LuxQ quorum-sensing receptor does not appear to form higher-order clusters [28]. If clustering of receptors provides added sensitivity to small changes in chemotaxis attractants, perhaps arranging quorum-sensing receptors as independent detectors protects the cell from responding to minor fluctuations in autoinducers. This arrangement likely allows cells to measure the slow build-up of autoinducers and at a critical concentration, commit to new behaviors that require a change in global transcription.

Future Questions and Goals

As technologies continue to improve our ability to monitor gene, protein, and metabolite levels in living cells, and as we find new ways to identify small molecules produced by bacteria, discovery of new quorum-sensing systems will likely accelerate. To fulfill the promise that an understanding of quorum sensing will allow us to manipulate bacterial behaviors by interfering with communication systems, autoinducer analogs and signaling inhibitors will need to be synthesized, or natural ones discovered and tested in vivo. Large chemical libraries are becoming available, and experimental screening for quorum-sensing antagonists is a promising route. Likewise, rational design of compounds based on known autoinducers, and screens for compounds occurring naturally in the environment, could be alternative resources of inhibitors. If quorum-sensing systems are susceptible to chemical inhibition, development of novel antimicrobial agents could ensue. With respect to AI-2-based quorum-sensing systems, LuxS appears to be a promising target for directed inhibitors because the LuxS crystal structure has been solved, all LuxS proteins synthesize the same moiety, DPD, and its enzymatic mechanism is defined. By interfering with production of the AI-2 signaling molecule, communication could be terminated in a diverse array of species

References

1. Nealson KH, Platt T, Hastings JW. Cellular control of the synthesis and activity of the bacterial luminescent system. J Bacteriol 1970;104:313–322. [PubMed: 5473898]
2. Fuqua C, Parsek MR, Greenberg EP. Regulation of gene expression by cell-to-cell communication: acyl-homoserine lactone quorum sensing. Annu Rev Genet 2001;35:439–468. [PubMed: 11700290]
3. Freeman JA, Lilley BN, Bassler BL. A genetic analysis of the functions of LuxN: a two-component hybrid sensor kinase that regulates quorum sensing in *Vibrio harveyi*. Mol Microbiol 2000;35:139–149. [PubMed: 10632884]
4. Freeman JA, Bassler BL. A genetic analysis of the function of LuxO, a two-component response regulator involved in quorum sensing in *Vibrio harveyi*. Mol Microbiol 1999;31:665–677. [PubMed: 10027982]
5. Lenz DH, Mok KC, Lilley BN, Kulkarni RV, Wingreen NS, Bassler BL. The small RNA chaperone Hfq and multiple small RNAs control quorum sensing in *Vibrio harveyi* and *Vibrio cholerae*. Cell 2004;118:69–82. [PubMed: 15242645]
6. Waters CM, Bassler BL. The *Vibrio harveyi* quorum-sensing system uses shared regulatory components to discriminate between multiple autoinducers. Genes Dev 2006;20:2754–2767. [PubMed: 17015436]
7. Bassler BL, Wright M, Silverman MR. Multiple signaling systems controlling expression of luminescence in *Vibrio harveyi*: sequence and function of genes encoding a second sensory pathway. Mol Microbiol 1994;13:273–286. [PubMed: 7984107]
8. Taga ME, Semmelhack JL, Bassler BL. The LuxS-dependent autoinducer AI-2 controls the expression of an ABC transporter that functions in AI-2 uptake in *Salmonella typhimurium*. Mol Microbiol 2001;42:777–793. [PubMed: 11722742]

9. Xavier KB, Bassler BL. Interference with AI-2-mediated bacterial cell-cell communication. Nature 2005;437:750–753. [PubMed: 16193054]
10. Schauder S, Shokat K, Surette MG, Bassler BL. The LuxS family of bacterial autoinducers: biosynthesis of a novel quorum-sensing signal molecule. Mol Microbiol 2001;41:463–476. [PubMed: 11489131]
11. Miller ST, Xavier KB, Campagna SR, Taga ME, Semmelhack MF, Bassler BL, Hughson FM. *Salmonella typhimurium* recognizes a chemically distinct form of the bacterial quorum-sensing signal AI-2. Mol Cell 2004;15:677–687. [PubMed: 15350213]
12. Chen X, Schauder S, Potier N, Van Dorsselaer A, Pelczer I, Bassler BL, Hughson FM. Structural identification of a bacterial quorum-sensing signal containing boron. Nature 2002;415:545–549. [PubMed: 11823863]
13. Day WA Jr, Maurelli AT. *Shigella flexneri* LuxS quorum-sensing system modulates virB expression but is not essential for virulence. Infect Immun 2001;69:15–23. [PubMed: 11119484]
14. Fong KP, Chung WO, Lamont RJ, Demuth DR. Intra- and interspecies regulation of gene expression by *Actinobacillus actinomycetemcomitans* LuxS. Infect Immun 2001;69:7625–7634. [PubMed: 11705942]
15. Lyon WR, Madden JC, Levin JC, Stein JL, Caparon MG. Mutation of *luxS* affects growth and virulence factor expression in *Streptococcus pyogenes*. Mol Microbiol 2001;42:145–157. [PubMed: 11679074]
16. Ohtani K, Hayashi H, Shimizu T. The *luxS* gene is involved in cell-cell signalling for toxin production in *Clostridium perfringens*. Mol Microbiol 2002;44:171–179. [PubMed: 11967077]
17. DeLisa MP, Wu CF, Wang L, Valdes JJ, Bentley WE. DNA microarray-based identification of genes controlled by autoinducer 2-stimulated quorum sensing in *Escherichia coli*. J Bacteriol 2001;183:5239–5247. [PubMed: 11514505]
18. Dove JE, Yasukawa K, Tinsley CR, Nassif X. Production of the signaling molecule, autoinducer-2, by *Neisseria meningitidis*: lack of evidence for a concerted transcriptional response. Microbiology 2003;149:1859–1869. [PubMed: 12855737]
19. Joyce EA, Kawale A, Censini S, Kim CC, Covacci A, Falkow S. LuxS is required for persistent pneumococcal carriage and expression of virulence and biosynthesis genes. Infect Immun 2004;72:2964–2975. [PubMed: 15102809]
20. Sperandio V, Torres AG, Giron JA, Kaper JB. Quorum sensing is a global regulatory mechanism in enterohemorrhagic *Escherichia coli* O157:H7. J Bacteriol 2001;183:5187–5197. [PubMed: 11489873]
21. Sztajer H, Lemme A, Vilchez R, Schulz S, Geffers R, Yip CY, Levesque CM, Cvitkovitch DG, Wagner-Dobler I. Autoinducer-2-regulated genes in *Streptococcus mutans* UA159 and global metabolic effect of the *luxS* mutation. J Bacteriol 2008;190:401–415. [PubMed: 17981981]
22. Hotchkiss RD. Cyclical behavior in pneumococcal growth and transformability occasioned by environmental changes. Proc Natl Acad Sci USA 1954;40:49–55. [PubMed: 16589433]
23. Luo P, Morrison DA. Transient association of an alternative sigma factor, ComX, with RNA polymerase during the period of competence for genetic transformation in *Streptococcus pneumoniae*. J Bacteriol 2003;185:349–358. [PubMed: 12486073]
24. Rickard AH, Palmer RJ Jr, Blehert DS, Campagna SR, Semmelhack MF, Egland PG, Bassler BL, Kolenbrander PE. Autoinducer-2: a concentration-dependent signal for mutualistic bacterial biofilm growth. Mol Microbiol 2006;60:1446–1456. [PubMed: 16796680]
25. Magnuson R, Solomon J, Grossman AD. Biochemical and genetic characterization of a competence pheromone from *B. subtilis*. Cell 1994;77:207–216. [PubMed: 8168130]
26. Bassler BL, Wright M, Showalter RE, Silverman MR. Intercellular signaling in *Vibrio harveyi*: sequence and function of genes regulating expression of luminescence. Mol Microbiol 1993;9:773–786. [PubMed: 8231809]
27. Neiditch MB, Federle MJ, Miller ST, Bassler BL, Hughson FM. Regulation of LuxPQ receptor activity by the quorum-sensing signal autoinducer-2. Mol Cell 2005;18:507–518. [PubMed: 15916958]

28. Neiditch MB, Federle MJ, Pompeani AJ, Kelly RC, Swem DL, Jeffrey PD, Bassler BL, Hughson FM. Ligand-induced asymmetry in histidine sensor kinase complex regulates quorum sensing. Cell 2006;126:1095–1108. [PubMed: 16990134]

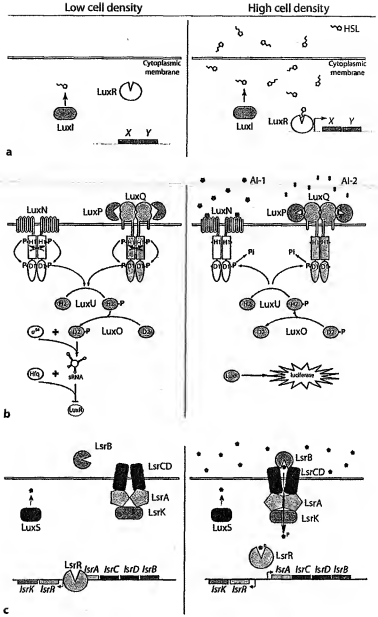


Fig. 1.

Quorum-sensing signaling networks. Low cell density (left sides of panels), High cell density (right sides of panels). **a** A LuxI/LuxR quorum-sensing circuit. The LuxI enzyme produces an AHL molecule that diffuses into the surroundings. At high cell densities, the LuxR protein binds the AHL and subsequently binds to DNA promoting the transcription of genes *X* and *Y*. **b** *V. harveyi* AI-1- and AI-2-dependent circuits. At low cell density, LuxN and LuxQ exist in kinase mode autophosphorylating at histidine (H1) and aspartic acid (D1) residues. Phosphate is passed to LuxU at the histidine (H2) site, and then to LuxO at an aspartic acid (D2). LuxO-P, with sigma factor σ^{54}, activates transcription of the sRNA genes. The sRNAs, with the RNA chaperone Hfq repress *luxR* expression. At high cell

densities, AI-1 binds LuxN and AI-2 binds LuxP-LuxQ. Binding of autoinducers causes LuxN and LuxQ to switch to phosphatase mode. Phosphate is drained from LuxU and LuxO, terminating expression of the sRNA genes. LuxR is derepressed, and luciferase is expressed. c The *E. coli* and *S. typhimurium* Lsr system. At low cell densities, LsrR binds to DNA and represses expression of the *lsr* operon. At high cell densities, AI-2 is imported via the Lsr transporter and is phosphorylated by LsrK. AI-2-phosphate binds to LsrR causing it to release DNA, thus derepressing *lsr* transcription [adapted from 8,28].

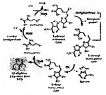


Fig. 2.
Reactive methyl cycle and production of DPD. SAM-dependent methyltransferases convert SAM to SAH, accumulation of which confers product-feedback inhibition on methyltransferase reactions. SAH is detoxified to SRH by Pfs. SRH is converted to homocysteine and DPD by LuxS. Homocysteine can be recycled to SAM via MetH, which generates methionine, and MetK.

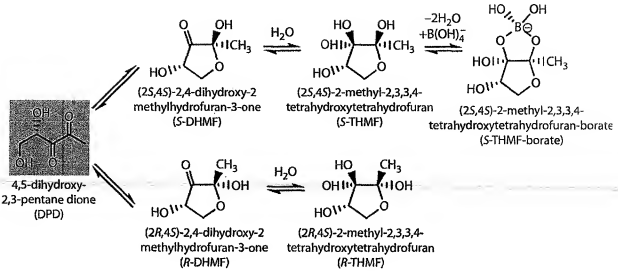


Fig. 3.
Interconverting forms of DPD. Cyclization of DPD generates two major stereoisomers *S*-DHMF and *R*-DHMF. Hydration in aqueous solution forms *S*-THMF and *R*-THMF. *S*-THMF is able to complex with borate, forming *S*-THMF-borate [adapted from 11].

Fig. 4.

Model for AI-2 dependent LuxPQ receptor activity. The top panels display the $LuxPQ_p$ receptor complex from a 'top-down' view, and the lower panels are a 90° rotation, showing the complex from the side. In the absence of AI-2 (left) LuxQ-LuxQ9 dimers exist in a symmetric orientation both in the periplasm and the cytoplasm, and therefore are in kinase mode. AI-2 binding to LuxP (right) causes a conformational change in LuxP, and induces interactions with LuxQ9. Simultaneous contacts of LuxP with LuxQ and LuxQ9 rotates the complex into an asymmetric orientation. Rotation of the periplasmic domains is conferred to the cytoplasmic domains, switching LuxQ from kinase to phosphatase mode [figure 4 reproduced with permission, 28].

Exhibit B

BLAST ®

Basic Local Alignment Search Tool

NCBI/ BLAST/ blastp suite-2sequences/ **Formatting Results - 6Z09NHHE114**

Formatting options

Download

Blast 2 sequences

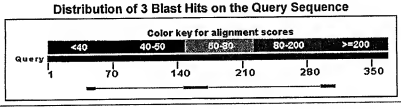
gb|AAA20837| (365 letters)

Query ID	gi|16082690|gb|AAA20837.2|
Description	ABC-type sugar transport system periplasmic protein [Vibrio harveyi ATCC BAA-1116] >gi|2143789|sp|P54300.2|LUXP_VIBHA RecName: Full=Autoinducer 2-binding periplasmic protein luxP; Flags: Precursor >gi|16082690|gb|AAA20837.2| LuxP [Vibrio harveyi] >gi|156528171|gb|ABU73256.1| hypothetical protein VIBHAR_05351 [Vibrio harveyi ATCC BAA-1116]
Molecule type	amino acid
Query Length	365

Subject ID	gi|107933356|gb|AAN86705.2|
Description	LuxR [Vibrio harveyi]
Molecule type	amino acid
Subject Length	205
Program	BLASTP 2.2.25+

Graphic Summary

Distribution of 3 Blast Hits on the Query Sequence



Dot Matrix View

Plot of gi|16082690|gb|AAA20837.2| vs gi|107933356|gb|AAN86705.2| [?]

This dot matrix view shows regions of similarity based upon the BLAST results. The query sequence is represented on the X-axis and the numbers represent the bases/residues of the query. The subject is represented on the Y-axis and again the numbers represent the bases/residues of the subject. Alignments are shown in the plot as lines. Plus strand and protein matches are slanted from the bottom left to the upper right corner, minus strand matches are slanted from the upper left to the lower right. The number of lines shown in the plot is the same as the number of alignments found by BLAST.

Descriptions

Legend for links to other resources: U UniGene E GEO G Gene S Structure M Map Viewer M PubChem BioAssay

Accession	Description	Max score	Total score	Query coverage	E value	Links
AAN86705.2	LuxR [Vibrio harveyi]	16.5	45.8	14%	0.72	M

Alignments

>gb|AAN86705.2| M LuxR [Vibrio harveyi]
Length=205

Sort alignments for this subject sequence by:
E value Score Percent identity
Query start position Subject start position

```
 Score = 16.5 bits (31),  Expect = 0.72, Method: Compositional matrix adjust.
 Identities = 6/10 (60%), Positives = 8/10 (80%), Gaps = 0/10 (0%)

Query  43   NLTNALSEAV  52
            N+TNA+ E V
Sbjct  95   NITNAMIELV  104


 Score = 15.4 bits (28),  Expect = 1.6, Method: Compositional matrix adjust.
 Identities = 8/30 (27%), Positives = 15/30 (50%), Gaps = 4/30 (13%)

Query  147  VLDSTNTKLLQNITTPVRE----WDKHQP  172
            V   +  +L++QN+      E     D+N+P
Sbjct  130  VTNRTNQLLVQNMFIKAIERGEVCDQHEP  159


 Score = 13.1 bits (22),  Expect = 6.8, Method: Compositional matrix adjust.
 Identities = 5/16 (31%), Positives = 8/16 (50%), Gaps = 0/16 (0%)

Query  294  AELDAIQKGDLDITVM  309
            AEL  +    LD+  +
Sbjct  184  AELSTLVSAYLDMLCI  199
```

BLAST ®

Basic Local Alignment Search Tool

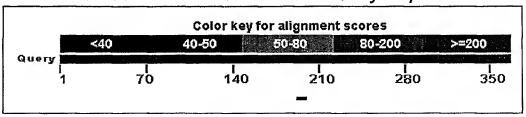
Blast 2 sequences

gb|AAA20837| (365 letters)

	Query ID	Subject ID
	gi|16082690|gb|AAA20837.2|	
Description	ABC-type sugar transport system periplasmic protein [Vibrio harveyi ATCC BAA-1116] >gi|21431789|sp|P54300.2|LUXP_VIBHA RecName: *Full=Autoinducer 2-binding* periplasmic protein luxP; Flags: Precursor >gi|16082690|gb|AAA20837.2| LuxP [Vibrio harveyi] >gi|156528171|gb|ABU73256.1| hypothetical protein VIBHAR_05351 [Vibrio harveyi ATCC BAA-1116]	gi|37223202|gb|AAQ90196. LuxR [Vibrio fischeri ES114]
Molecule type	amino acid	amino acid
Query Length	365	
Subject Length		230
Program		BLASTP 2.2.25+

Graphic Summary

Distribution of 1 Blast Hits on the Query Sequence



Plot of gi|16082690|gb|AAA20837.2| vs gi|37223202|gb|AAQ90196.1|

This dot matrix view shows regions of similarity based upon the BLAST results. The query sequence is represented on the X-axis and the numbers represent the bases/residues of the query. The subject is represented on the Y-axis and again the numbers represent the bases/residues of the subject. Alignments are shown in the plot as lines. Plus strand and protein matches are slanted from the bottom left to the upper right corner, minus strand matches are slanted from the upper left to the lower right. The number of lines shown in the plot is the same as the number of alignments found by BLAST.

Accession	Description			Links
	LuxR [Vibrio fischeri ES114]	14.2	2%	3.9

>gb|AAQ90196.1| LuxR [Vibrio fischeri ES114]
Length=230

Score = 14.2 bits (25), Expect = 3.9, Method: Compositional matrix adjust.
Identities = 5/8 (63%), Positives = 5/8 (63%), Gaps = 0/8 (0%)

```
Query  192  GKFFPKHT  199
            G  FP HT
Sbjct  121  GFSFPIHT  128
```

BLAST ®

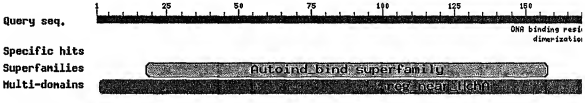
Basic Local Alignment Search Tool

LasR

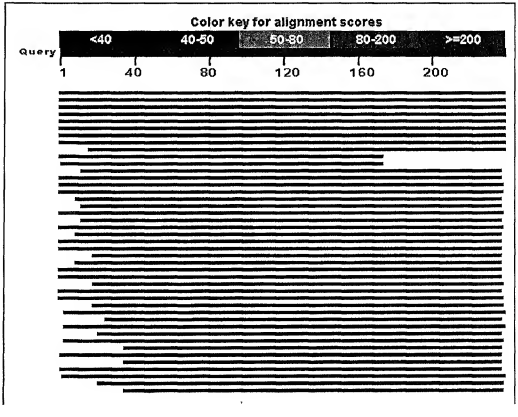
Query ID	lcl|91808	**Database Name**	nr
Description	None	**Description**	All non-redundant GenBank CDS translations+PDB+SwissProt+P excluding environmental samples from WGS projects
Molecule type	amino acid		
Query Length	239	**Program**	BLASTP 2.2.26+

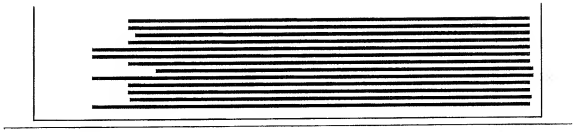
Graphic Summary

Putative conserved domains have been detected, click on the image below for detailed results.



Distribution of 100 Blast Hits on the Query Sequence





Descriptions

Legend for links to other resources: U UniGene E GEO G Gene S Structure M Map Viewer PubChem BioAssay

Accession	Description	Max score	Total score	Query coverage	E value	Max ident	Links
BAA06489.1	LasR [Pseudomonas aeruginosa]	496	496	100%	9e-179	100%	
NP_250121.1	lasR gene product [Pseudomonas aeruginosa PAO1] >ref|YP_791822.1| lasR gene product [Pseudomonas aeruginosa UCBPP-PA14] >ref|YP_002441567.1| lasR gene product [Pseudomonas aeruginosa LESB58] >ref|ZP_04927852.1| transcriptional regulator LasR [Pseudomonas aeruginosa C3719] >ref|ZP_04933102.1| transcriptional regulator LasR [Pseudomonas aeruginosa 2192] >ref|ZP_07792658.1| transcriptional regulator LasR [Pseudomonas aeruginosa 39016] >ref|ZP_09054016.1| hypothetical protein HMPREF1030_03102 [Pseudomonas sp. 2_1_26] >sp|P25084.1|LASR_PSEAE RecName: Full=Transcriptional activator protein lasR >gb|AAG04819.1|AE004572_7 transcriptional regulator LasR [Pseudomonas aeruginosa PAO1] >gb|AAA25874.1| transcriptional activator LasR [Pseudomonas aeruginosa PAO1] >gb|ABJ10611.1| transcriptional regulator LasR [Pseudomonas aeruginosa UCBPP-PA14] >gb|EAZ51971.1| transcriptional regulator LasR [Pseudomonas aeruginosa C3719] >gb|EAZ57221.1| transcriptional regulator LasR [Pseudomonas aeruginosa 2192] >gb|ABU49653.1| LasR [Pseudomonas sp. M18] >emb|CAW28738.1| transcriptional regulator LasR [Pseudomonas aeruginosa LESB58] >gb|EFQ37754.1| transcriptional regulator LasR [Pseudomonas aeruginosa 39016] >gb|AEO76182.1| transcriptional regulator LasR [Pseudomonas aeruginosa M18] >gb|EHF13072.1| hypothetical protein HMPREF1030_03102 [Pseudomonas sp. 2_1_26]	495	495	100%	3e-178	99%	G
AAT50467.1	PA1430 [synthetic construct]	495	495	100%	3e-178	99%	
BAA06490.1	LasR [Pseudomonas aeruginosa PA103]	492	492	100%	4e-177	99%	

Accession	Description	Max score	Total score	Query coverage	E value	Max ident	Links
GAA16961.1	transcriptional regulator LasR [Pseudomonas aeruginosa NCMG1179]	491	491	100%	9e-177	99%	
YP_001349253.1	transcriptional regulator LasR [Pseudomonas aeruginosa PA7] >gb|ABR86383.1| transcriptional regulator LasR [Pseudomonas aeruginosa PA7]	491	491	100%	2e-176	98%	G
CAO85750.1	LasR protein [Pseudomonas aeruginosa]	481	481	100%	7e-173	97%	
BAK89171.1	transcriptional regulator [Pseudomonas aeruginosa NCGM2.S1]	476	476	100%	6e-171	97%	
ZP_01364783.1	hypothetical protein PaerPA_01001895 [Pseudomonas aeruginosa PACS2] >ref|ZP_06879878.1| transcriptional regulator LasR [Pseudomonas aeruginosa PAb1] >gb|EGM20008.1| transcriptional regulator LasR [Pseudomonas aeruginosa 138244] >gb|EGM20464.1| transcriptional regulator LasR [Pseudomonas aeruginosa 152504]	464	464	93%	3e-166	99%	
3IX3_A	Chain A, Lasr-Oc12 Hsl Complex >pdb|3IX3|B Chain B, Lasr-Oc12 Hsl Complex >pdb|3IX4|A Chain A, Lasr-Tp1 Complex >pdb|3IX4|B Chain B, Lasr-Tp1 Complex >pdb|3IX4|C Chain C, Lasr-Tp1 Complex >pdb|3IX4|D Chain D, Lasr-Tp1 Complex >pdb|3IX4|E Chain E, Lasr-Tp1 Complex >pdb|3IX4|F Chain F, Lasr-Tp1 Complex >pdb|3IX4|G Chain G, Lasr-Tp1 Complex >pdb|3IX4|H Chain H, Lasr-Tp1 Complex >pdb|3IX8|A Chain A, Lasr-Tp3 Complex >pdb|3IX8|B Chain B, Lasr-Tp3 Complex >pdb|3IX8|C Chain C, Lasr-Tp3 Complex >pdb|3IX8|D Chain D, Lasr-Tp3 Complex >pdb|3JPU|A Chain A, Lasr-Tp4 Complex >pdb|3JPU|B Chain B, Lasr-Tp4 Complex >pdb|3JPU|C Chain C, Lasr-Tp4 Complex >pdb|3JPU|D Chain D, Lasr-Tp4 Complex >pdb|3JPU|E Chain E, Lasr-Tp4 Complex	358	358	72%	3e-125	99%	S
2UV0_E	Chain E, Structure Of The P. Aeruginosa Lasr Ligand-Binding Domain Bound To Its Autoinducer >pdb|2UV0|F Chain F, Structure Of The P. Aeruginosa Lasr Ligand-Binding Domain Bound To Its Autoinducer >pdb|2UV0|G Chain G, Structure Of The P. Aeruginosa Lasr Ligand-Binding Domain Bound To Its	347	347	71%	8e-121	98%	S

BLAST ®

Basic Local Alignment Search Tool

NCBI/ BLAST/ blastp suite-2sequences/ **Formatting Results - J5D6WU1G11N**

Formatting options

Download

Blast 2 sequences

gi|541657 (239 letters)

Query ID	gi|541657|dbj|BAA06489.1|	**Subject ID**	gi|321225366|gb|EFX50424.1
Description	LasR [Pseudomonas aeruginosa]	**Description**	LsrR, transcriptional repressor of lsr operon [Salmonella enterica subsp. enterica serovar Typhimurium str. TN061786]
Molecule type	amino acid	**Molecule type**	amino acid
Query Length	239	**Subject Length**	319
		Program	BLASTP 2.2.26+

Descriptions

Alignments

No significant similarity found. For reasons why, click here.